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Hematology of healthy Florida manatees (*Trichechus manatus*)

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Key Words
Heinz bodies, hematology, reticulocyte, manatees, schistocyte, stomatocyte

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Background: Hematologic analysis is an important tool in evaluating the general health status of free-ranging manatees and in the diagnosis and monitoring of rehabilitating animals.
Objectives: The purpose of this study was to evaluate diagnostically important hematologic analytes in healthy manatees (*Trichechus manatus*) and to assess variations with respect to location (free ranging vs captive), age class (small calves, large calves, subadults, and adults), and gender.
Methods: Blood was collected from 55 free-ranging and 63 captive healthy manatees. Most analytes were measured using a CELL-DYN 3500R; automated reticulocytes were measured with an ADVIA 120. Standard manual methods were used for differential leukocyte counts, reticulocyte and Heinz body counts, and plasma protein and fibrinogen concentrations.
Results: Rouleaux, slight polychromasia, stomatocytosis, and low numbers of schistocytes and nucleated RBCs (NRBCs) were seen often in stained blood films. Manual reticulocyte counts were higher than automated reticulocyte counts. Heinz bodies were present in erythrocytes of most manatees. Compared with free-ranging manatees, captive animals had slightly lower MCV, MCH, and eosinophil counts and slightly higher heterophil and NRBC counts, and fibrinogen concentration. Total leukocyte, heterophil, and monocyte counts tended to be lower in adults than in younger animals. Small calves tended to have higher reticulocyte counts and NRBC counts than older animals.
Conclusions: Hematologic findings were generally similar between captive and free-ranging manatees. Higher manual reticulocyte counts suggest the ADVIA detects only reticulocytes containing large amounts of RNA. Higher reticulocyte and NRBC counts in young calves probably reflect an increased rate of erythropoiesis compared with older animals.

Introduction

The Florida manatee (*Trichechus manatus*) is an endangered aquatic mammal living in the coastal oceans, rivers, and estuaries of the southeastern United States, primarily in Florida. The long-term survival of manatees is dependent on protecting habitat, recognizing the potential impact of the loss of man-made warm water refuges, understanding the involvement of biotoxins, and minimizing human-induced injury and death. The most common causes of death are blunt and cutting trauma from watercraft collisions, perinatal disorders, acute and chronic hypothermia, infectious diseases, and inhalation and/or ingestion of brevetoxins produced by red tide dinoflagellates (*Karenia brevis*).[1,2] Some disorders are related. For example, trauma and chronic hypothermia can lead to secondary bacterial or fungal infections.[3]

Ill and injured, free-ranging animals are rescued and rehabilitated at oceanaria and marine parks and preserves and, whenever possible, released when deemed healthy by experienced manatee veterinarians utilizing physical examination and hematology, clinical chemistry, and serum amyloid A (SAA) test results. Environmental conditions and diet differ between the wild and captive settings.[4]

Hematologic analysis is an important tool in surveys of general health status of free-ranging manatees and in the diagnosis and monitoring of rehabilitating animals. To identify occult disease and to interpret analyte values from sick manatees, laboratory-specific reference intervals for each analyte should be established from a large sample of healthy animals. A previous study of plasma biochemical analytes revealed many significant differences based on age and gender and between free-ranging and captive animals.[4] The primary purpose of this study was to determine whether significant hematologic differences were present in apparently healthy manatees with regard to location (free ranging vs captive), age class, or gender. A secondary goal was to better characterize the morphology of the erythrocytes, leukocytes, and platelets of manatees. Although the number of animals studied was not sufficient to establish definitive reference intervals for some of the analytes measured, this information can be used to develop working reference intervals. In addition, differences between free-ranging and captive manatees might suggest management issues for consideration in species recovery efforts.

Materials and Methods

Animals and sample collection

This project was approved by the University of Florida Institutional Animal Care and Use Committee (approval #D882). Blood samples were collected under United States Fish and Wildlife Service Permits #MA773494-8 (Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute) and #MA791721-4 (US Geological Survey, Sirenia Project) and assayed under United States Fish and Wildlife Service Permit #MA067116-0 (University of Florida, Aquatic Animal Health Program). Blood was collected from the brachial vascular bundle (also known as the pectoral arteriovenous plexus) directly into potassium EDTA anticoagulant tubes during routine health assessments of apparently healthy free-ranging manatees ($n = 55$) during captures performed by the Florida Fish and Wildlife Conservation Commission and the US Geological Survey, Sirenia Project between 2002 and 2006. Blood was similarly collected from healthy captive manatees housed at SeaWorld Orlando ($n = 24$), Lowry Park Zoo ($n = 28$), Miami Seaquarium ($n = 3$), Cincinnati Zoo ($n = 3$), Epcot's Living Seas ($n = 3$), and Mote Marine Laboratory ($n = 2$) from 2002 to 2006. Free-ranging manatees were captured in nets deployed either from shore or from a specialized net boat in open water.[5] Blood samples were collected throughout the year, but most samples from free-ranging animals were collected during December (44%) and January (27%), when manatees congregate in springs and warm-water discharge areas near power plants. Most of the free-ranging animals (72%) were captured in the Tampa Bay, Florida, area, with 20% captured between Sarasota, Florida, and Naples, Florida; 4% captured in the Florida Everglades, and 4% captured in the St. John's River of northeast Florida.

After collection, blood samples were stored on wet ice or in a refrigerator at 4°C until assayed at the University of Florida, Veterinary Medical Center, Clinical Pathology Laboratory. When possible, blood samples were analyzed within 6 hours of collection; however, all samples were assayed within 24 hours. To determine whether time delay had an effect, blood samples from 24 manatees were analyzed on the day of sample collection and then again 1 day later.

Animals were included in the study if they were considered to be healthy on the basis of physical examination, were not visibly pregnant, and had SAA concentrations ≤ 50 mg/L. Increased SAA concentration has been shown to be a reliable indicator of systemic inflammation in manatees.[6]

Manatees were assigned to 1 of 4 age groups using body length (standard straight length) measurements established by the US Geological Survey's Sirenia Project for adults (≥ 265 cm), subadults (236–264 cm), large calves (200–235 cm), and small calves (< 200 cm). Based on location (free ranging or captive) and age classification, the following sample sizes were obtained: 34 free-ranging and 28 captive adults, 10 free-ranging and 25 captive subadults, 8 free-ranging and 9 captive large calves, and 3 free-ranging and 1 captive small calves.

Laboratory analysis

Erythrocyte and platelet parameters and total leukocyte counts were determined using an automated hematology analyzer (CELL-DYN 3500R with Veterinary Package Software, Abbott Laboratories, Abbott Park, IL, USA). The software allowed for user-defined manatee settings to be established by adjusting a preset species (elephants) with similar cellular characteristics. Blood films were made at the University of Florida, and blood films were also made at the site of collection for most animals. Blood films were examined after staining with Wright–Giemsa (Harleco, EMD Chemicals Inc., Gibbstown, NJ, USA), and 200-cell manual leukocyte differential counts were performed. Nucleated RBCs (NRBCs) were enumerated during the differential leukocyte count and the absolute NRBC count was determined by multiplying the NRBC/100 WBC ratio times the total WBC count. Reticulocytes and Heinz bodies were identified and counted manually using a microscope after staining supravitally with new methylene blue (N Brecher, Harleco, EMD Chemicals Inc.) and expressed as a percentage of 1000 mature RBCs. Automated reticulocyte counts were also determined, using an ADVIA 120 hematology system with multispecies software (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA). The ADVIA software allowed for user-defined manatee settings to be established by adjusting a preset species (humans) with similar cellular characteristics.

Total plasma protein concentration was measured using a refractometer (Reichert VET 360, Reichert Analytical Instruments Inc., Depew, NY, USA), and fibrinogen concentration was estimated using a heat precipitation method.[7] SAA concentration was measured using a manual antibovine ELISA (Tridelta Development Limited, Maynooth, Ireland) validated for use in manatee plasma.[6] The lower limit of detection was 10 mg/L. Serum cortisol concentration was measured in archived serum samples (frozen at $-80\,^{\circ}\mathrm{C}$ for up to 5 years) from 27 free-ranging and 14 captive manatees using an automated chemiluminescent analyzer (IMMULITE 1000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) and a polyclonal rabbit anticortisol immunoassay (product no. LKC01, Siemens Medical Solutions Diagnostics). Analytical validation of this system using manatee serum was completed at the University of Florida College of Veterinary Medicine Endocrinology Laboratory to ensure cross-reactivity of antibodies to cortisol in manatee serum.[8]

Transmission electron microscopy

To compare ultrastructural characteristics of eosinophil and heterophil granules, fresh blood was collected in EDTA from 3 healthy manatees with high-normal numbers of blood eosinophils; samples were centrifuged at low speed to pack the cells. Plasma was removed by pipette and replaced with 2.5% glutaraldehyde in 0.1-M phosphate buffer, pH 7.4, without disturbing the buffy coat. The samples were fixed overnight at 4°C, washed in 0.1-M phosphate buffer, postfixed in 2% osmium tetroxide for 1 hour, dehydrated in ethanol, and embedded in an epon-araldite mixture. One-micrometer toluidine blue-stained sections of buffy coats were evaluated to select areas for thin sectioning. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined using a Philips CM 100 electron microscope (Philips Electron Optics, Eindhoven, the Netherlands); images were acquired using an AMT HR digital camera (Advanced Microscopy Techniques Corp., Danvers, MA, USA).

Statistical analysis

Statistical analyses were performed using SigmaStat (Systat Software Inc., Richmond, CA, USA). A paired *t*-test was used to compare 2 samples from the same animal if the data passed a Kolmogorov–Smirnov test for normality. If not, the Wilcoxon signed-rank test was used for paired samples. Two-way ANOVA with Tukey's test for comparison of pairs was used to compare test results from all manatees (except small calves) by location (free ranging vs captive) and blood sample age (same day or day old), as well as by location and animal age. A *t*-test was used to compare reticulocyte counts in fresh blood samples of free-ranging vs captive animals. Two-way ANOVA was also used to compare gender and location in adult animals (29 females and 33 males). Data from small calves were not included in 2-way ANOVA assays, but were included in age comparisons using a 1-way ANOVA on ranks test with Dunn's test for comparison of pairs. Bivariant simple linear regression was used to determine the correlation between manual and automated reticulocyte counts. The total number of manatees evaluated for most analytes was 118, but fewer ($n = 81$) were analyzed for manual reticulocyte counts and Heinz body counts. Pairs were considered statistically different when $P < .05$.

Results

Several features of manatee blood cells were recognized during examination of stained blood films (Figures 1 and 2). Low numbers of polychromatophilic erythrocytes were seen (Figure 1a) and mild to moderate rouleaux and stomatocytosis were frequently observed (Figure 1b). Additionally, low numbers of schistocytes (Figure 1c), NRBCs (Figure 2h), echinocytes, (Figure 1d), and Howell–Jolly bodies (Figure 1e and f) were seen. Indistinct pale inclusions were sometimes observed in erythrocytes (Figure 1c–e) that were found to be Heinz bodies in new methylene blue-stained preparations (Figure 1f–h). Platelets were small, with an overall mean platelet volume (MPV) of

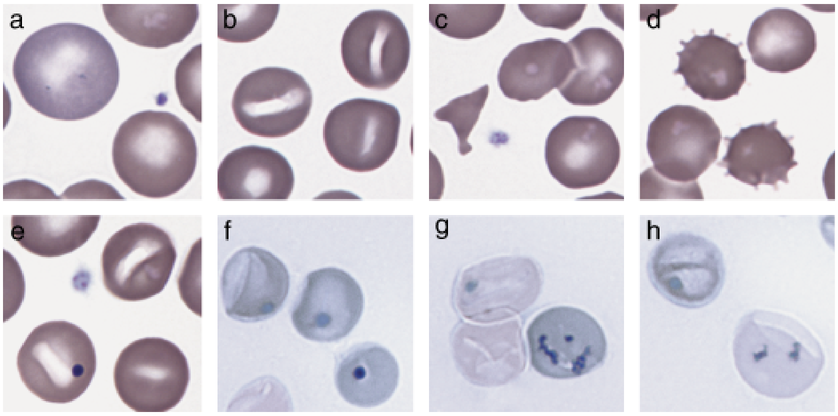

Figure 1. Erythrocyte morphology in manatee blood. (**a**) Large polychromatophilic erythrocyte, mature erythrocyte, and small platelet. (**b**) Three stomatocytes and a discocyte. (**c**) A triangular schistocyte, 2 erythrocytes at upper right that each contains a pale Heinz body and a small platelet. (**d**) Two echinocytes that each contains a pale Heinz body. (**e**) Stomatocytes, 1 contains a Howell–Jolly body (bottom) and 1 contains a Heinz body (top). A small platelet is also present. (**f**) Two erythrocytes at the top contain light-blue Heinz bodies; an erythrocyte at the bottom contains a Howell–Jolly body. (**g**) A reticulocyte containing substantial aggregated dark-blue ribosomal material and an erythrocyte containing a light-blue Heinz body. (**h**) A reticulocyte containing scant aggregated dark-blue ribosomal material and an erythrocyte containing a light blue Heinz body. (**a–e**) Wright–Giemsa (**f–h**) new methylene blue, $\times$100 objective.

6.4 fL $\pm$ 0.8 (Figure 1a, c, and e). Small platelet aggregates were frequently seen, especially in blood films prepared from day-old blood.

Manatees had heterophils (rather than neutrophils) (Figure 2a) with pale blue cytoplasm and round, oval, or rod-shaped pink to red granules. Eosinophils had larger, round, more refractile, darker red granules and darker blue cytoplasm than heterophils (Figure 2b). Nuclei of eosinophils were generally less segmented than those of mature heterophils and often were band shaped. Most lymphocytes were small to medium in size (Figure 2e and f) and low numbers of granular lymphocytes were observed (Figure 2g). Monocyte morphology was similar to that seen in domestic mammals (Figure 2d). Like eosinophils, basophils often had band-shaped nuclei. Basophils had purple granules that varied in number, size, and shape (Figure 2c).

By transmission electron microscopy (TEM), heterophils contained many variably sized granules that were often elongated (Figure 3). Eosinophils contained fewer, larger, more densely stained granules than heterophils did (Figure 4).

Slight but significant increases in mean HCT (0.359–0.364 L/L) and hemoglobin concentration (113–115 g/L) were observed in day-old samples compared with results at 6 hours postcollection (P = .006 and P < .001, respectively). Mean total plasma protein

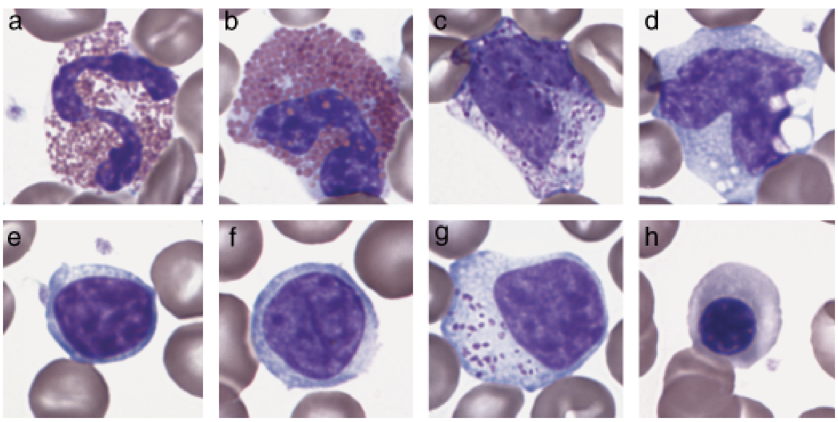

Figure 2. Morphology of nucleated cells in manatee blood. (**a**) Heterophil. (**b**) Eosinophil with band-shaped nucleus. (**c**) Basophil with band-shaped nucleus. (**d**) Monocyte. (**e**) Small lymphocyte. (**f**) Medium-sized lymphocyte. (**g**) Large granular lymphocyte. (**h**) Nucleated RBC (polychromatophilic rubricyte). Wright–Giemsa, $\times$100 objective.

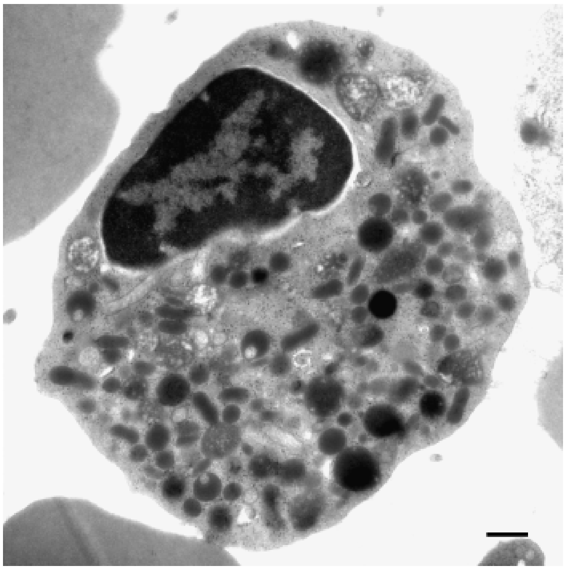

Figure 3. Transmission electron micrograph of a manatee heterophil containing many variably sized and variably shaped granules. A few large, more densely stained granules may be primary granules, with smaller less densely stained granules being secondary granules. Uranyl acetate and lead citrate stain. Bar = 500 nm.

concentrations also increased significantly from 78.5 to 80.4 g/L (P=.004). There was a slight but significant decrease in mean platelet count from 285×10^9 to 256×10^9 L (P=.015) and a slight increase in MPV from 5.82 to 6.28 fL ($P < .001$). Mean total WBC count decreased from 8.28×10^9 to 7.37×10^9 L ($P < .001$) and was primarily attributable to a decrease in mean lymphocyte count from 3.11×10^9 to 2.55×10^9 L (P=.002). Day-old blood samples were analyzed from 40 captive animals and 23 free-ranging animals. Consequently, sample age was included in statistical analysis using a 2-way ANOVA when comparing results by location (free ranging vs captive).

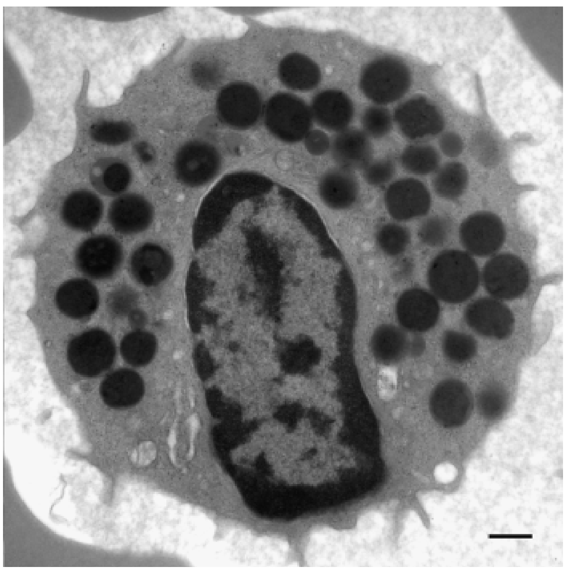

Figure 4. Transmission electron micrograph of a manatee eosinophil containing large, round, densely stained granules. Uranyl acetate and lead citrate stain. Bar = 500 nm.

Table 1. Hematologic results for free-ranging and captive manatees (*Trichechus manatus*).*

Analyte	Free Ranging (n = 52)	Captive (n = 62)	P Value
HCT (L/L)	0.351 (0.289–0.435)	0.350 (0.277–0.461)	.731
Hemoglobin (g/L)	112 (94–135)	112 (86–149)	.784
RBC count ($\times 10^{12}$/L)	2.76 (2.17–3.39)	2.81 (2.30–3.51)	.366
MCV (fL)	128 (114–140)	124 (105–140)	.003
MCH (pg)	40.8 (36.6–44.9)	40.0 (32.9–44.3)	.015
MCHC (g/L)	320 (280–354)	320 (294–336)	.973
Red cell distribution width (%)	16.2 (13.9–22.8)	16.6 (13.5–21.4)	.139
Platelets ($\times 10^9$/L)	242 (111–424)	274 (137–507)	.051
Mean platelet volume (fL)	6.44 (5.02–8.49)	6.40 (5.19–8.80)	.625
WBC count ($\times 10^9$/L)	5.98 (2.77–13.50)	6.64 (2.85–14.2)	.070
Bands ($\times 10^9$/L)	0.02 (0–0.22)	0.02 (0-0.16)	.912
Heterophils ($\times 10^9$/L)	2.33 (0.77–6.53)	3.25 (1.40–8.50)	$< .001$
Lymphocytes ($\times 10^9$/L)	2.90 (1.01–7.20)	2.71 (0.83–8.50)	.811
Monocytes ($\times 10^9$/L)	0.51 (0.08–1.70)	0.56 (0.07–2.80)	.537
Eosinophils ($\times 10^9$/L)	0.20 (0–1.23)	0.09 (0–0.41)	$< .001$
Basophils ($\times 10^9$/L)	0.04 (0–0.27)	0.03 (0–0.14)	.138
Nucleated RBC ($\times 10^9$/L)	0.02 (0–0.21)	0.04 (0–0.25)	.028
Reticulocytes, manual ($\times 10^9$/L)†	51 (16–96)	74 (0–127)	.076
Reticulocytes, automated ($\times 10^9$/L)‡	17 (7–45)	21 (3–39)	.424
Heinz bodies (%)§	8.2 (0.6–22.3)	7.0 (0–15)	.181
Plasma proteins (g/L)	80 (69–93)	81 (70–92)	.641
Fibrinogen (g/L)	2.24 (1.00–5.00)	3.23 (1.00–6.00)	.002

*Data are mean (minimum–maximum). Data for small calves were not included. Except for reticulocytes, P values were determined by comparing location (free ranging vs captive) and age of blood sample (fresh vs day old) using a 2-way ANOVA with the Tukey test for group comparisons. Only fresh blood samples were analyzed for reticulocyte counts, so P values were determined for free-ranging vs captive animals using a t-test.
†n = 17 free-ranging, 15 captive.
‡n = 10 free-ranging, 9 captive.
§n = 28 free-ranging, 40 captive.

Several small, but significant, differences were found when hematologic values from healthy captive and free-ranging manatees were compared (Table 1). The MCV was significantly higher in free-ranging compared with captive animals (P=.008), and there was a significant age difference (P=.025), with large calves having significantly lower values than adults (Figure 5). Low values could mainly be attributed to young captive

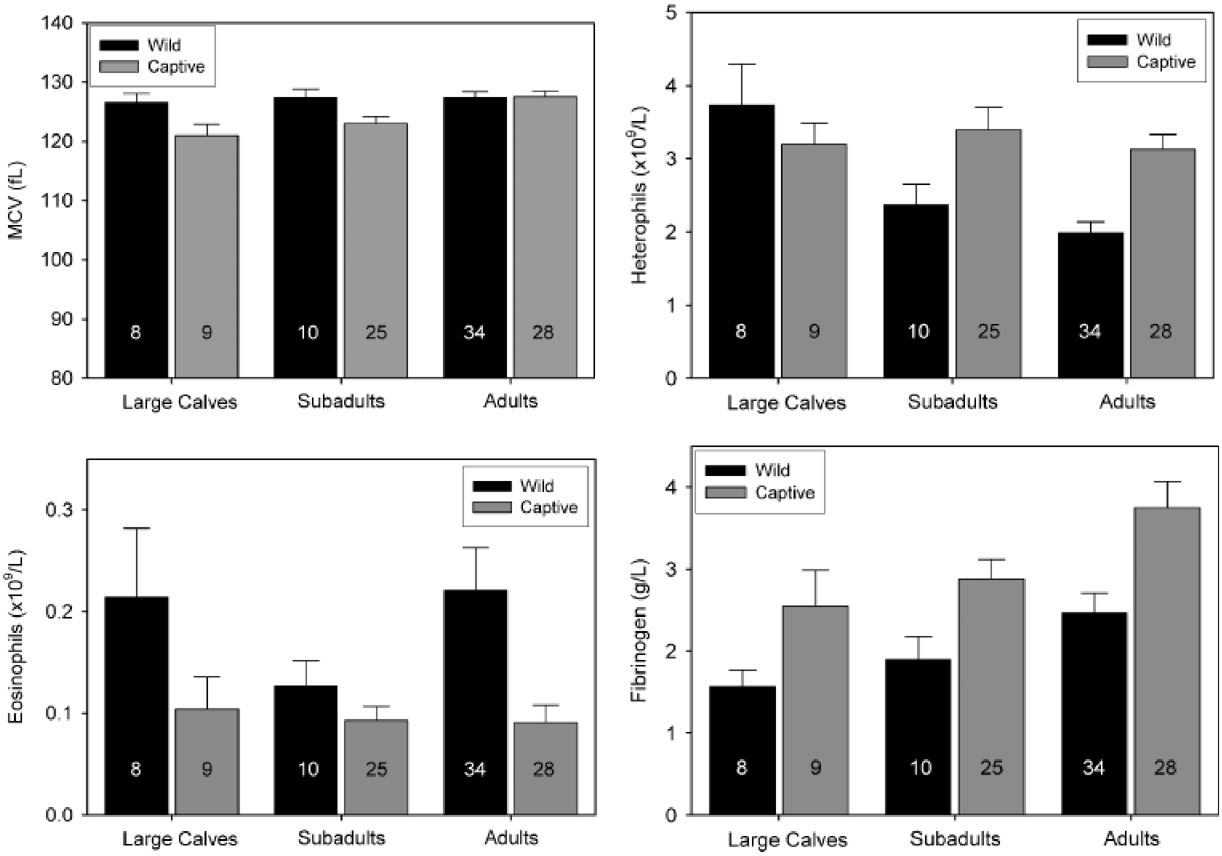

Figure 5. MCV, heterophil counts, eosinophil counts, and fibrinogen concentrations in free-ranging and captive manatees of different ages. See text for statistical differences between groups. Bars are mean ± 1 SEM. Numbers on bars indicate number of animals in each group.

manatees, but the interaction of location and age was not significant (P=.072). Heterophil counts were significantly lower in free-ranging compared with captive animals (P=.033), and a significant age difference (P=.015) and location X age interaction (P=.028) were found, with significantly higher values in free-ranging large calves compared with subadults and adults (Figure 5). Captive animals had lower eosinophil counts (P=.011), but there was no significant age difference (P=.409) (Figure 5). Fibrinogen concentrations were consistently higher in captive compared with free-ranging manatees ($P < .001$) and increased with age (P=.007) (Figure 5).

Forty-five (39%) healthy manatees had low numbers of NRBCs, and mean NRBC counts were slightly higher in captive compared with free-ranging manatees (Table 1). Except for 1 captive animal, all manatees had a low percentage (<15%) of Heinz bodies (Table 1). No difference in Heinz body percentage was found in captive vs free-ranging animals.

The amount of blue aggregated material (presumably RNA) in reticulocytes varied considerably (Figure 1G and H). Although there was no significant difference between captive and free-ranging animals, the manual reticulocyte percentage (3.0 ± 1.6%) and counts ($80 \pm 41 \times 10^9$ L) on day-old blood were higher (P=.014 and P=.021, respectively) than those obtained within 6 hours of collection (2.1 ± 1.0%, $57 \pm 27 \times 10^9$ L). This was largely attributed to a lower reticulocyte count in fresh blood samples from free-ranging animals (Figure 6). Automated reticulocyte counts were also measured in 48 animals in this study. Automated reticulocyte counts (n=48) were significantly higher in free-ranging animals ($P < .001$) and day-old blood ($P < .001$). There was also a statistically significant interaction between location and sample age ($P < .001$). Automated reticulocyte counts were especially high in day-old blood from free-ranging manatees (Figure 6). There was no significant correlation between manual and automated reticulocyte percentages (r=.22, P=.134).

Few gender differences were observed between adult male and female manatees. Males had slightly higher HCT (0.354 ± .027 vs 0.338 ± .027 L/L, P=.021)

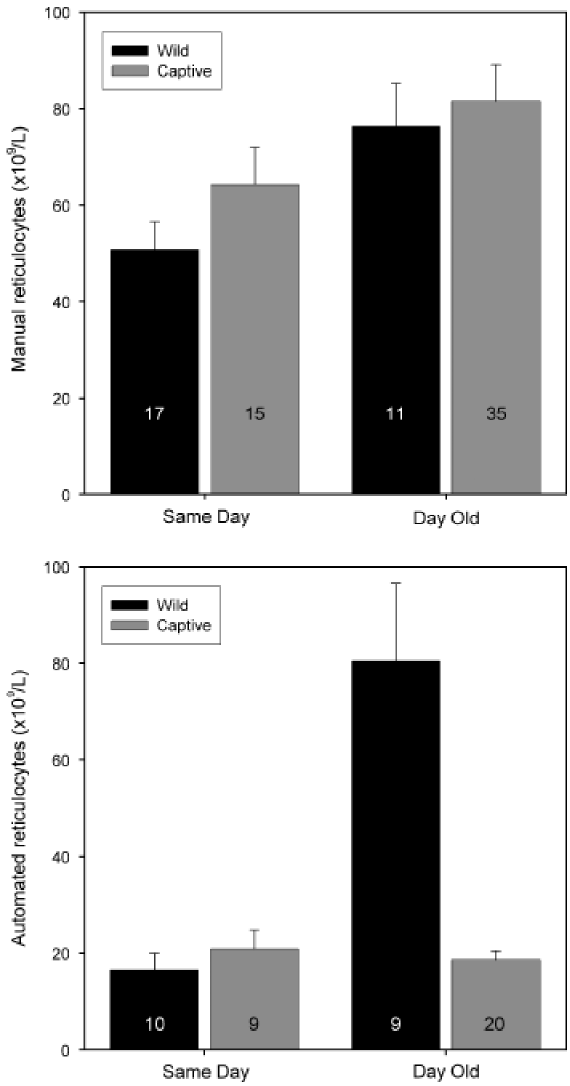

Figure 6. Manual and automated reticulocyte counts in free-ranging (wild) and captive manatees performed within 6 hours of sample collection or 1 day later. See text for statistical differences between groups. Bars are mean ± 1 SEM. Numbers on bars indicate number of animals in each group.

and MPV ($6.75 \pm .79$ vs $6.16 \pm .77$ fL, P=.011) than females. Mean hemoglobin concentrations were also higher in males (113 ± 9 g/L) compared with females (109 ± 9 g/L), but the differences were not significant (P=.082).

Age comparisons were limited by the number of animals in each age group, especially young calves. Total leukocyte, heterophil, lymphocyte, and monocyte counts tended to be lower in adults than in younger animals (Figure 7), but the differences were not significant for lymphocyte counts. Small calves had higher reticulocyte counts and higher NRBC counts than older animals (Figure 8).

There was no significant difference (P=.16) in cortisol concentration between captive (10 ± 5, 3–19 nmol/L) and free-ranging (13 ± 7, 1–33 nmol/L) manatees.

Discussion

The primary purpose of this study was to determine whether significant hematologic differences were present in healthy manatees with regard to location (free ranging vs captive), age class, or gender. This required that hematologic analytes be measured from many more animals than in past studies. A secondary goal was to better characterize the morphology of all blood cell types in manatees. Most hematologic findings in this study were similar to those in previously published manatee studies.[9–13] HCT and hemoglobin concentration were similar to those of domestic mammals and other marine mammals.[13] Like other marine mammals, manatees in this and other studies have larger erythrocytes and lower erythrocyte counts than domestic mammals.[13] Platelet counts were similar to values in domestic mammals. Platelets are small, with MPVs comparable to those of cattle and horses.

Except for the recognition that erythrocytes are large and low numbers of NRBCs may be present,[10,11] morphologic features of erythrocytes in stained blood films from healthy manatees have not been described previously. Polychromasia was absent or slight as has been reported in marine mammals in general.[13] Rouleaux was usually seen, although it was generally less pronounced than in horses or cats. The stomatocytosis and echinocytosis may have been artifacts of blood handling and blood film preparation and drying, but the low numbers of schistocytes would be difficult to attribute to these mechanisms. Removal of erythrocyte fragments by the spleen may be less robust in manatees than in other mammals. This possibility is supported by the occurrence of low numbers of Heinz bodies in erythrocytes of most healthy manatees.

Leukocyte counts tended to be slightly lower than those of most domestic mammals, but were similar to those in multiple dolphin and whale species.[13] Florida manatees have heterophils (pink to red granules) rather than neutrophils (nearly colorless granules). Heterophils from manatees stain positively for myeloperoxidase, like neutrophils in domestic mammals.[14] This is in contrast to heterophils from rabbits and birds that do not stain positively for myeloperoxidase.[14] Heterophils and lymphocytes were usually present in approximately equal numbers in manatees. Lymphocyte morphology was similar to that of other

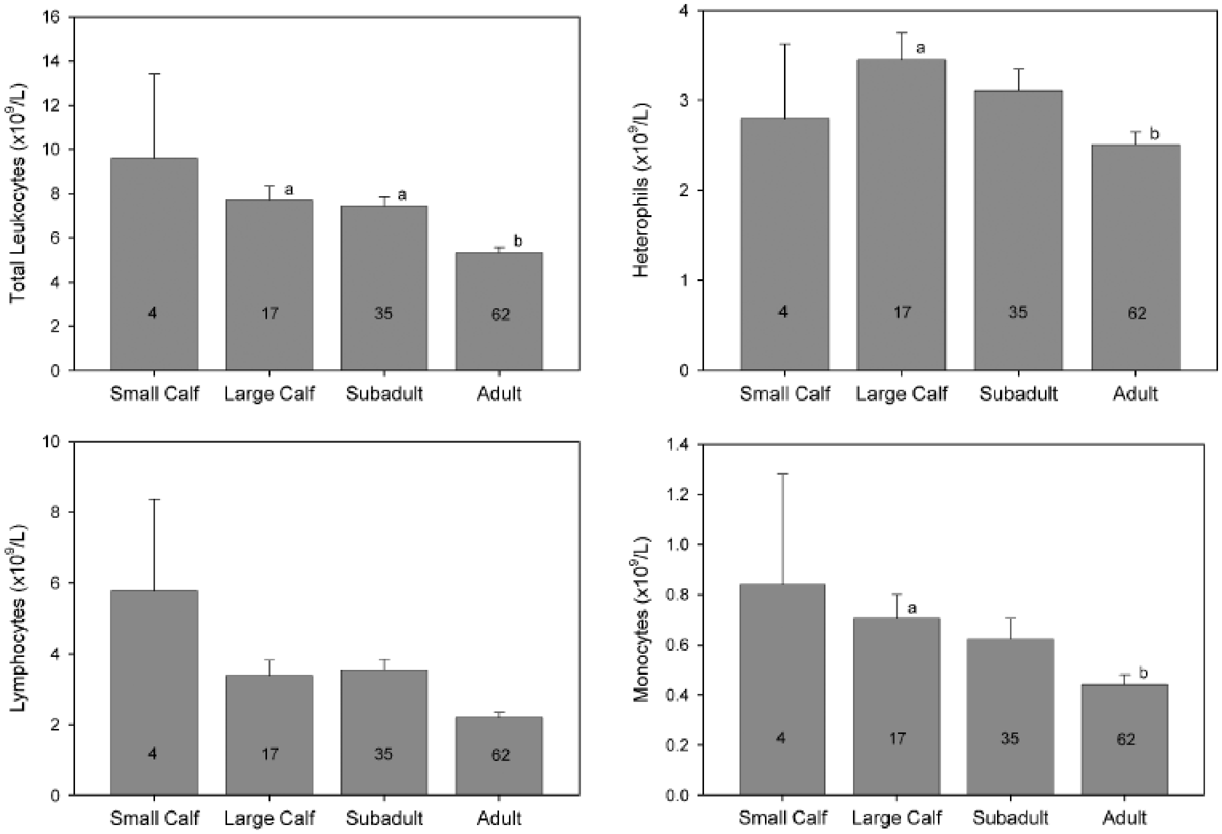

Figure 7. Total leukocyte, heterophil, lymphocyte, and monocyte counts shown by age group. Number of manatees in each group is indicated on bar. Bars are mean ± 1 SEM. Groups with different letters are significantly different ($P < .05$). For lymphocyte counts, there were no significant differences between age groups.

mammals. In contrast to counts for other blood cells, lymphocyte counts decreased in day-old blood, indicating that these cells are susceptible to lysis (based on morphology in stained blood films) when stored at 5°C for 1 day. The low numbers of large granular lymphocytes may be cytotoxic T lymphocytes or natural killer cells, as have been reported in other mammalian species.

Eosinophil and basophil counts have been reported as zero in some recent publications on healthy Florida manatees,[12,13] likely because of a failure to differentiate eosinophils from heterophils, which also have red granules. In other studies, low numbers of eosinophils and basophils were found in some, but not all, manatees.[10,15] In contrast, we found eosinophils in 85% of manatees and basophils in 49% of manatees in this study. Although we were able to differentiate heterophils from eosinophils in Wright–Giemsa-stained blood films, a Luna stain for eosinophils is reported to facilitate this differentiation.[10] Eosinophils and heterophils were easily differentiated by TEM based on the morphology of their granules. Granules with crystalline structures are seen in eosinophils from humans and many animal species; however, granules in manatee eosinophils were generally homogenous, as has been reported in eosinophils from cattle, gorillas, and mink.[16] Basophils are not heavily granulated in manatees and might be missed, especially if Diff-Quik stain is used.

Low numbers of NRBCs were observed in 40% of the manatees in this study. Low numbers of NRBCs have been reported in some previous studies of healthy manatees,[10,11] but not in others.[9,13] Healthy manatees normally have extramedullary hematopoiesis in the spleen, liver, kidney, and vertebrae.[17] Extramedullary erythropoiesis (especially in the spleen) might explain the common occurrence of NRBCs in these animals. NRBCs are reported to be increased in the blood of manatees with chronic inflammatory conditions.[13] If true, the higher number of NRBCs in captive manatees might be attributable to a higher frequency of occult inflammatory disorders in captive compared with free-ranging manatees.

Heinz bodies have not been reported previously in healthy manatees. In this study, Heinz bodies were

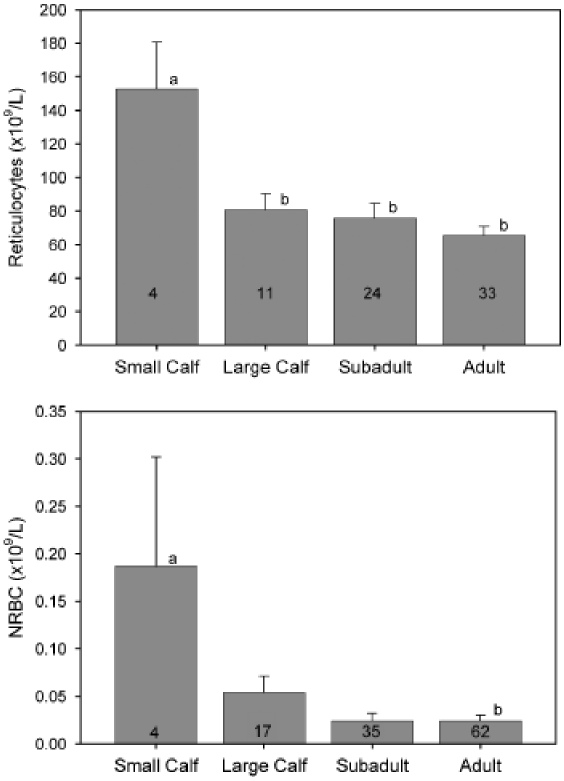

Figure 8. Manual reticulocyte and nucleated RBC (NRBC) counts shown by age group. Numbers of manatees in each group are indicated on bars. Bars are mean ± 1 SEM. Groups with different letters are significantly different ($P < .05$).

sometimes recognized as pale inclusions in Wright–Giemsa-stained blood films but were more clearly seen and quantified using the new methylene blue stain. The presence of Heinz bodies in erythrocytes from healthy cats has been attributed to an increased susceptibility of feline hemoglobin to oxidative denaturation and to poor pitting function (removal of Heinz bodies) by the feline spleen.[18,19] It is not known whether either of these features explain the presence of Heinz bodies in manatees.

An early study in which dried blood films were stained with new methylene blue indicated that normal manatees lacked reticulocytes,[10] but in a later study, in which supravital new methylene blue staining was used, uncorrected reticulocyte percentages of 0–4% were reported.[13] Manual reticulocyte counts were considerably higher than automated reticulocyte counts in our study, suggesting that the ADVIA 120 was only able to identify reticulocytes with large amounts of RNA in their cytoplasm. In cats, the ADVIA counts correlate with the number of aggregate reticulocytes, but not punctate reticulocytes (J.W. Harvey, unpublished observations). The presence of only slight polychromasia in stained blood films indicated that most reticulocytes in manatees contained insufficient RNA to produce a blue color in these cells. Although the amount of aggregated blue material ("reticulin") varied considerably in manatee reticulocytes, punctate reticulocytes were not appreciated. The higher reticulocyte counts in day-old blood are difficult to explain. Although storage of blood might in some way promote precipitation of RNA by new methylene blue, this would not explain the much higher automated reticulocyte counts in day-old blood compared with fresh blood from free-ranging manatees.

Several small but significant differences were documented when hematologic values from free-ranging and captive manatees were compared. Mean MCV and MCH values were slightly lower in young captive manatees compared with young free-ranging manatees. The cause(s) of these subtle differences is unknown, but differences in iron metabolism should be considered. The slightly higher heterophil counts in captive animals might be the result of occult, localized inflammation, greater basal endogenous glucocorticoid secretion, or transiently increased glucocorticoid secretion associated with restraint of multiple animals in holding areas for up to a day before blood sample collection. While the initial restraint may be more vigorous in the capture of free-ranging manatees, blood sampling may occur sooner than for many of the animals bled in oceanaria. Although epinephrine released during excitement or exercise may also increase heterophil counts, one might expect a higher (rather than lower) heterophil count in free-ranging manatees because the restraint process is more active in free-ranging compared with captive animals. Glucocorticoids can result in increased neutrophil counts and decreased lymphocyte and eosinophil counts in domestic animals. Although the mean lymphocyte count was not significantly lower in captive animals, the higher mean heterophil count and lower mean eosinophil count in captive compared with free-ranging manatees suggested a glucocorticoid effect. However, there was no significant difference in serum cortisol concentration between these groups. Fecal cortisol levels were reported to be significantly higher in 1 small group of captive manatees (not included in this study) compared with free-ranging manatees in Florida.[20] Based on these conflicting findings, an additional prospective study of cortisol metabolism in captive vs free-ranging manatees is warranted.

There was no significant difference in SAA concentration between groups, indicating that changes in fibrinogen concentration were not due to systemic inflammation. The higher mean fibrinogen concentration in captive animals might be due to increased glucocorticoid concentration, as has been reported in pigs and humans.[21,22] Captive animals in this study were housed in oceanaria for months to years for rehabilitation after disease or injury. Although they were considered healthy based on physical examination and normal SAA results, it is possible that some had occult, localized inflammation; however, serum cortisol concentrations also did not support this.

Total leukocyte, heterophil, and monocyte counts were generally lower in adults than in younger animals. Although HCT was not significantly lower in small calves, they had significantly higher reticulocyte and NRBC counts than older animals. This may reflect increased erythropoiesis required for blood volume expansion during growth as well as replacement of aged, fetal erythrocytes removed by the mononuclear phagocyte system. Adult males had slightly higher HCT and MPV than females. This sex difference also occurs in humans and is attributable to serum testosterone concentrations.[23,24]

In conclusion, our evaluation indicates that blood from healthy manatees contains rouleaux formation, slight polychromasia, and low numbers of schistocytes, Heinz bodies, and NRBCs. In contrast to previous reports, eosinophils and basophils are often present in low numbers. The ADVIA hematology analyzer underestimates reticulocyte counts in manatees. Significant differences based on captivity, gender, and age class should be considered in the interpretation of hematologic results from manatees.

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